Effect of Dietary Protein and Exogenous Gonadotropin-Releasing Hormone on Circulating Progesterone Concentrations and Performance of Holstein Cows[1]

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ABSTRACT

Sixty-three Holstein cows were used in a 2 × 2 factorial arrangement of treatments to determine the effects of dietary protein and exogenous gonadotropin-releasing hormone on performance postpartum and on circulating concentrations of progesterone. The experimental diets, 14 or 20% CP (dry basis), were fed from parturition to 14 wk postpartum. Blood serum urea N was elevated in the cows fed the 20% versus 14% CP diet. Feed intake was increased, but yield and composition of milk were not affected by the higher protein diet. Circulating concentrations of progesterone were lower in cows fed 20% than 14% protein on d 12 of both the synchronized and subsequent estrous cycles. Exogenous gonadotropin-releasing hormone administered on d 12 of the preceding estrous cycle did not increase the concentration of progesterone on d 12 of the subsequent estrous cycle. Luteal phase concentrations of progesterone were reduced by high dietary protein, but were not affected by gonadotropin-releasing hormone given on d 12 of the preceding cycle.

INTRODUCTION

Higher amounts of CP have been fed to cows to improve milk production by increasing feed consumption and digestibility. In reviews on protein nutrition (4, 19), the authors con-cluded that intakes of protein that maximize milk yield are detrimental to reproduction. Ferguson et al. (7) compiled data from seven studies and generally found that increasing dietary CP content was associated with increases in services per conception and days open. The mechanism by which high dietary protein might be detrimental to reproduction is not known. Several studies cited by Ferguson et al. (7) indicated that higher dietary CP increased rumen and blood ammonia and blood urea N. Two hypotheses have been proposed: 1) excess ammonia absorbed from the rumen produces biochemical, endocrinological, and tissue derangements; and 2) additional absorbed protein alters the balance of net protein and net energy to cause a relative energy deficiency (4). The suggested sites at which excessive dietary protein might inhibit reproductive processes include: the ovary, hypothalamus, pituitary, or uterus (7, 8, 14, 15).

Fertility of cattle inseminated at a natural estrus is related to the endocrine profile before and after insemination. Britt and Holt (3) indicated that higher concentrations of progesterone in the blood during the luteal phase of the estrous cycle preceding insemination were associated with higher conception rates. Progesterone concentrations were lower (9, 15) or not affected (2) in dairy cows fed high protein diets.

Administration of a gonadotropin-releasing hormone (GnRH) analog to repeat breeder cows on d 12 of the estrous cycle resulted in a higher embryo recovery rate and increased the percentage of normal embryos compared to untreated control cows (18). The authors speculated that such a treatment 10 d prior to the following ovulation might have resulted in improved follicular recruitment for ovulation leading putatively to increased progesterone secretion by the corpus luteum.

The present study was designed to test whether elevated dietary protein and exogenous GnRH affected circulating concentrations of progesterone during the luteal phase of the estrous cycle. A second objective was to determine whether elevated dietary protein affected milk production and feed intake.

MATERIALS AND METHODS

Sixty-three Holstein cows were blocked by parity (primiparous or multiparous) and randomly assigned at parturition to a 2 × 2 factorial arrangement of treatments in a randomized block design. The two factors were dietary protein (14% CP and 20% CP, dry basis) and administration of exogenous GnRH (Control = no treatment; GnRH = 100 μg i.m.).

Cows were fed individually in a tie-stall barn from parturition to 14 wk postpartum. Isocaloric complete mixed diets of 22.5% ammoniated corn silage, 22.5% alfalfa haylage, and 55% concentrate mix, on a DM basis (72% TDN; net energy lactation = 1.52 Mcal/kg), were fed once daily. Protein content was adjusted by substituting soybean meal for sorghum grain in the concentrate mix.

Feed consumption was recorded twice weekly and the mean of these observations used to estimate average daily DM intake. Body weights were recorded weekly. Cows were milked and yield of milk recorded twice daily. Average daily production was summarized on a weekly basis. Milk composition was determined once monthly in samples collected at two consecutive milkings.

Cows were exercised and observed specifically for estrus twice daily for 30 min at 0700 and 1500 h. They also were observed casually for signs of estrus during movement to and from the milking parlor and during routine management procedures.

Only cows between 30 and 60 d postpartum with a palpable corpus luteum were administered prostaglandin $F_{2\alpha}$ (The Upjohn Co., Kalamazoo, MI) to synchronize estrus. On d 12 after the synchronized estrus, the assigned cows were given 100 μg GnRH (Abbott Laboratories, Chicago, IL) injected i.m. Blood samples were collected from 34 cows that were at the proper stage postpartum and that exhibited a synchronized estrus. Blood samples were collected via jugular venipuncture or from a coccygeal vessel in a vacutainer tube immediately prior to GnRH treatment. Thereafter blood samples were collected on alternate days until d 12 (range, d 10 to 14) of the subsequent estrous cycle when a serial bleeding was conducted. Blood was collected via jugular catheter at 30-min intervals for 9 h to establish the circulating concentrations of progesterone. Blood samples were placed on ice immediately after collection and serum harvested 48 h later. A composite serum sample was prepared for each cow by pooling equal aliquots from the 19 individual samples collected from each cow. The composite samples were stored frozen until analyzed. Progesterone data from the 34 cows that satisfied the criteria were used in the statistical analyses.

Concentrations of progesterone in the serum were determined by double antibody radioimmunoassay (Serono Diagnostics, Braintree, MA). Sensitivity of the assay was .1 ng/ml, and intraassay and interassay coefficients of variation were 9 and 11%, respectively. Serum urea nitrogen (SUN) was determined (16) in samples collected at the beginning and end of the intensive bleeding period to confirm that the dietary treatments affected N metabolism.

Data were analyzed using the General Linear Models procedure of the SAS (11) for least squares. The model for analysis of circulating concentrations of progesterone and SUN concentrations included dietary CP, GnRH treatment, parity, and interactions. Milk production and composition, body weight, and DM intake were evaluated by split-plot analysis (17) in which the factorial arrangement of diet in parity group was considered to be the whole plot effect and week of lactation was considered to be the subplot. Treatment differences were tested utilizing the methods of Gill (10) for analyzing data involving nonrandom repeated measurements.

RESULTS

Performance data are given in Table 1. Blood SUN was increased ($P<.0001$) in cows fed the diet containing 20% CP compared with those fed the 14% CP diet. This confirms that the dietary treatments had produced effects on N metabolism. Feed intake was increased

TABLE 1. Effect of dietary protein on postpartum performance.[1]

	Dietary crude protein			
	14%		20%	
Number of cows	31		32	
	$\overline{X}$	SE	$\overline{X}$	SE
Serum urea N, mg/dl	12.70[a]	.80	29.30[b]	.30
Feed intake, dry basis, kg/d	18.70[c]	.20	20.10[d]	.20
Lactational responses				
Milk yield, kg/d	25.80	.22	27.10	.22
Milk fat, %	3.68	.05	3.72	.06
Milk protein, %	3.01	.02	3.02	.02

[a,b]Means within a row with different superscripts differ ($P<.0001$).

[c,d]Means within a row with different superscripts differ ($P<.03$).

[1]Least square means and standard errors.

($P<.03$) by the 20% CP diet. The increase in milk yield was not significant. Milk composition was unchanged ($P>.05$). Feed intake (21.2 ± .2 vs. 17.6 ± .2 kg/d) and milk yield (29.8 ± .2 vs. 23.1 ± .2 kg/d) were greater ($P<.0001$) in multiparous than in primiparous cows. The overall changes in body weight throughout the trial were small and were unaffected by dietary treatment. However, cows fed 20% CP lost more weight during the first 5 wk of lactation.

Circulating concentrations of progesterone were higher (Table 2, $P<.05$) in cows fed the 14% CP diet than those fed the 20% CP diet on d 12 of the synchronized estrous cycle and during the midluteal phase of the subsequent estrous cycle (4.91 vs. 3.47 ng/ml and 4.61 vs. 3.33 ng/ml, respectively). Treating cows with exogenous GnRH on d 12 of the synchronized estrous cycle did not significantly increase the circulating concentrations of progesterone during the midluteal phase of the subsequent estrous cycle (3.46 vs. 4.48 ng/ml for control vs. GnRH, respectively). The concentration of progesterone in response to the GnRH treatment tended ($P<.10$) to be greater for cows fed the 14% CP diet than those fed the 20% CP diet (Table 2).

DISCUSSION

Concentrations of SUN (12.7 and 29.3 mg/dl for the 14% and 20% CP diets, respectively) were associated positively with dietary intake of CP in the present study. This is in agreement with data from previous reports (1, 13). Concentrations of SUN below 10 mg/dl are thought to indicate a protein deficiency in cattle (12), and conception rate declined in cows with SUN above 20 mg/dl (7). Therefore, the protein content of the low protein diet was apparently adequate and the high protein diet was excessive with regard to concentrations of SUN. Thus, the protein amounts fed resulted in concentrations of SUN desired in the present study.

Dry matter intake was increased but milk yield and composition were unchanged in cows fed the 20% CP compared with cows fed the 14% CP. These results are similar to the findings of Blauwiekel and Kincaid (1). Howard et al. (13), comparing 20 and 15% protein diets, reported enhanced milk yield, but daily DM intake was unaffected by the 20% diet. Little or no response in milk yield to increased dietary protein was observed where there was no change in energy intake (6). Nutritional stress, as indicated by body weight change, was small and unaffected by dietary protein.

Circulating concentrations of progesterone were depressed during the midluteal phase of the estrous cycle in cows fed 20% compared with cows fed 14% CP. These results are similar to the findings of Jordan and Swanson (15) and Folman et al. (9) but contrary to those of Blauwiekel et al. (2). The reasons for these different findings are not apparent. Although SUN was not reported in the three trials cited, Hammond (12) reported that SUN is affected by feed intake and physiological state or stage

TABLE 2. Effect of dietary protein and gonadotropin-releasing hormone treatments on concentrations of progesterone in blood serum.

	14% CP				20% CP			
	Control		GnRH		Control		GnRH	
	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE	$\overline{X}$	SE
Progesterone, ng/ml								
d 12, 1st cycle	4.91[a]	.46			3.47[b]	.50		
d 12, 2nd cycle	3.81[c]	.62	5.41[d]	.69	3.12[c]	.65	3.54[c]	.74

[a,b]Means within a row with different superscripts differ $(P<.05)$.

[c,d]Means within a row with different superscripts differ $(P<.10)$.

[1]Means derived from a single blood sample per cow collected on d 12 of the synchronized estrous cycle immediately prior to GnRH treatment (n = 34 cows).

[2]Means derived from one pooled blood sample per cow collected on d 12 (range 10 to 14) of the estrous cycle subsequent to the cycle of GnRH treatment. The pooled sample for each cow was formed from equal aliquots from 19 blood samples collected at 30-min intervals over a 9-h period (n = 34 cows).

of production in addition to the protein content of the diet. Cows in early lactation were used in the studies that observed depressed progesterone concentrations with a high protein diet (9, 15, present study), whereas nonlactating cows were used in the study by Blauwiekel et al. (2). Average daily DM intake in the present study was approximately threefold higher than those observed for nonlactating cows (2). In a review, Chesworth and Easdon (5) concluded that underfeeding tends to increase progesterone in sheep, but the effects in cattle are equivocal. In some studies (3, 9), circulating progesterone concentrations in lactating dairy cows have been shown to be related positively to body weight gain and feed intake (9).

Thibier et al. (18) reported that administering a GnRH analogue on d 12 of the preceding estrous cycle resulted in a higher embryo recovery rate and a higher percentage of normal embryos in treated compared to control repeat breeder cows. The authors speculated the GnRH treatment 10 d prior to the subsequent ovulation improved follicular recruitment for ovulation leading putatively to a corpus luteum that secreted more progesterone. Results of the present study do not support this speculation. Administration of GnRH during the preceding cycle did not increase the circulating concentrations of progesterone during the luteal phase of the subsequent cycle, although the increase approached significance in the cows fed the 14% CP protein diet.

In summary, high dietary protein elevated SUN concentrations and reduced circulating concentrations of progesterone in cows during early lactation. Administration of GnRH during the preceding luteal phase did not alter the concentration of progesterone during the subsequent luteal phase nor was this response significantly affected by the dietary treatment.

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